

V. CLAIMS

We claim:

- 1 An improved flow cytometer system for isolating desired cells comprising:
 - a. a cell source which supplies cells to be analyzed by the flow cytometer;
 - 5 b. a sheath fluid source which creates a sheath fluid environment for said cells which contains about 2.9% sodium citrate;
 - c. a nozzle through which said cells pass while subjected to said sheath fluid environment;
 - d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;
 - 10 e. a cell sensing system which responds to said cells;
 - f. a sorter discrimination system which acts to sort cells having a desired characteristic; and
 - g. a collector into which cells having a desired characteristic are placed.
- 2 An improved flow cytometer system for isolating desired cells as described in claim 1 wherein said cell source comprises bovine sperm cells.
- 3 An improved flow cytometer system for isolating desired cells comprising:
 - a. a cell source which supplies cells to be analyzed by the flow cytometer;
 - b. a sheath fluid source which creates a sheath fluid environment for said cells which contains a hepes buffered medium;
 - 20 c. a nozzle through which said cells pass while subjected to said sheath fluid environment;
 - d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;
 - e. a cell sensing system which responds to said cells;
 - 25 f. a sorter discrimination system which acts to sort cells having a desired characteristic; and

g. a collector into which cells having a desired characteristic are placed.

4 An improved flow cytometer system for isolating desired cells as described in claim 3 wherein said cell source comprises equine sperm cells.

5 An improved flow cytometer system for isolating desired cells comprising:

- 5
- a. a cell source which supplies cells to be analyzed by the flow cytometer;
 - b. a sheath fluid source which creates a sheath fluid environment for said cells;
 - c. a nozzle through which said cells pass while subjected to said sheath fluid environment;
 - d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;
 - e. a cell sensing system which responds to said cells;
 - f. a sorter discrimination system which acts to sort cells having a desired characteristic; and
 - g. a collector into which cells having a desired characteristic are placed and which comprises a citrate collector fluid containing about six percent egg yolk.
- 10
- 15

6 An improved flow cytometer system for isolating desired cells as described in claim 5 wherein said sheath fluid source comprises a solution containing about 2.9% sodium citrate.

7 An improved flow cytometer system for isolating desired cells as described in claim 5 or 6 wherein said cell source comprises bovine sperm cells.

20 8 An improved flow cytometer system for isolating desired cells comprising:

- a. a cell source which supplies cells to be analyzed by the flow cytometer;
- b. a sheath fluid source which creates a sheath fluid environment for said cells;
- c. a nozzle through which said cells pass while subjected to said sheath fluid environment;

- d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;
- e. a cell sensing system which responds to said cells;
- f. a sorter discrimination system which acts to sort cells having a desired characteristic; and
- 5 g. a collector into which cells having a desired characteristic are placed and which comprises a test tube having the physical characteristics of a stream-matched container.

9 An improved flow cytometer system for isolating desired cells as described in claim 8 wherein said cell source comprises cells which are mechanically delicate.

10 10 An improved flow cytometer system for isolating desired cells as described in claim 1, 3, or 5 wherein said cell source comprises cells which are hyper-responsive to a chemical composition in a surrounding fluid environment.

11 An improved flow cytometer system for isolating desired cells as described in claim 1, 3, 5, or 8 wherein said collector is used to provide a low dose of sperm.

12 An improved flow cytometer system for isolating desired cells as described in claim 11 wherein said nozzle, oscillator, cell sensing system, and sorter discrimination system are part of a flow cytometer system and wherein said flow cytometer system comprises a high speed cell sorter.

13 An improved flow cytometer system for isolating desired cells as described in claim 8 wherein said nozzle, oscillator, cell sensing system, and sorter discrimination system are part of a flow cytometer system and wherein said flow cytometer system comprises a high speed cell sorter.

14 A sexed sperm specimen produced according to a system as described in any of claims 1, 3, 5, 6, or 8, 22, 30, 34, 76, 78, 59, 72.

- 15 A sexed sperm specimen as described in claim 14 wherein said collector is used to provide a low dose of sperm.
- 16 A sexed sperm specimen as described in claim 14 wherein said nozzle, oscillator, cell sensing system, and sorter discrimination system are part of a flow cytometer system and wherein said flow cytometer system comprises a high speed cell sorter.
- 17 A sexed sperm specimen as described in claim 15 wherein said collector is used to provide a low dose of sperm.
- 18 An animal produced through use of a sexed sperm specimen produced according to a system as described in any of claims 1, 3, 5, 6, or 8, 22, 30, 34, 76, 78, 59, 72.
- 19 An animal as described in claim 18 wherein said animal is produced through use of a low dose of sperm.
- 20 An animal as described in claim 18 wherein said nozzle, oscillator, cell sensing system, and sorter discrimination system are part of a flow cytometer system and wherein said flow cytometer system comprises a high speed cell sorter.
- 21 An animal as described in claim 19 wherein said animal is produced through use of a low dose of sperm.
- 22 An improved flow cytometer system for isolating desired cells comprising:
- a. a cell source which supplies cells to be analyzed by the flow cytometer;
 - b. a chemically coordinated sheath fluid source which creates a sheath fluid environment for said cells which is selected to be coordinated with both a pre-sort and a post-sort cell fluid environment;

- c. a nozzle through which said cells pass while subjected to said sheath fluid environment;
- d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;
- e. a cell sensing system which responds to said cells;
- f. a sorter discrimination system which acts to sort cells having a desired characteristic; and
- g. a collector into which cells having a desired characteristic are placed.

23 An improved flow cytometer system for isolating desired cells as described in claim 22 wherein said pre-sort and post-sort cell fluid environments contain at least one hyper-responsive chemical composition to which said cells are particularly responsive and wherein said chemically coordinated sheath fluid source minimizes changes to said hyper-responsive chemical composition.

24 An improved flow cytometer system for isolating desired cells as described in claim 23 wherein said hyper-responsive chemical composition comprises a metabolic chemical composition.

25 An improved flow cytometer system for isolating desired cells as described in claim 23 wherein said hyper-responsive chemical composition comprises a citrate.

26 An improved flow cytometer system for isolating desired cells as described in claim 22 wherein said cell source creates said pre-sort cell fluid environment and wherein said collector creates said post-sort cell fluid environment.

27 An improved flow cytometer system for isolating desired cells as described in claim 23 wherein said cell source comprises non-repairing cells.

- 28 An improved flow cytometer system for isolating desired cells as described in claim 27 wherein said cell source comprises cells which have non-transcribing DNA.
- 29 An improved flow cytometer system for isolating desired cells as described in claim 27 wherein said cell source comprises cells which have non-replicating DNA.
- 5 30 An improved flow cytometer system for isolating desired cells as described in claim 27 wherein said cell source comprises sperm cells.
- 31 An improved flow cytometer system for isolating desired cells as described in claim 23 or 25 wherein said cell source comprises bovine sperm cells.
- 32 An improved flow cytometer system for isolating desired cells as described in claim 23 wherein said cell source comprises equine sperm cells.
- 33 An improved flow cytometer system for isolating desired cells as described in claim 22 wherein said cell source comprises cells which are hyper-responsive to a chemical composition in a sheath fluid environment.
- 34 An improved flow cytometer system for isolating desired cells as described in claim 30 wherein said collector is used to provide a low dose of sperm.
- 35 An improved flow cytometer system for isolating desired cells as described in claim 34 wherein said low dose of sperm comprises a dosage of less than about ten percent of said typical dosage.
- 36 An improved flow cytometer system for isolating desired cells as described in claim 34 wherein said sperm cells comprise bovine sperm cells and wherein said low dose of sperm comprises a dosage of less than about five hundred thousand sperm.
- 20

37 An improved flow cytometer system for isolating desired cells as described in claim 34 wherein said sperm cells comprise bovine sperm cells and wherein said low dose of sperm comprises a dosage of less than about three hundred thousand sperm.

38 An improved flow cytometer system for isolating desired cells as described in claim 34 wherein said sperm cells comprise equine sperm cells and wherein said low dose of sperm comprises a dosage of less than about ten million sperm.

39 An improved flow cytometer system for isolating desired cells as described in claim 25 wherein said chemically coordinated sheath fluid source comprises a solution containing about 2.9% sodium citrate.

40 An improved flow cytometer system for isolating desired cells as described in claim 39 wherein said cell source comprises bovine sperm cells.

41 An improved flow cytometer system for isolating desired cells comprising:

- a. a cell source which supplies cells to be analyzed by the flow cytometer;
- b. a sheath fluid source which creates a sheath fluid environment for said cells which comprises a solution containing about 2.9% sodium citrate;
- c. a nozzle through which said cells pass while subjected to said sheath fluid environment;
- d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;
- e. a cell sensing system which responds to said cells;
- f. a sorter discrimination system which acts to sort cells having a desired characteristic; and
- g. a collector into which cells having a desired characteristic are placed.

42 An improved flow cytometer system for isolating desired cells as described in claim 40 wherein said collector is used to provide a sperm for artificial insemination.

43 An improved flow cytometer system for isolating desired cells as described in claim 40 wherein said collector is used to provide a low dose of sperm for artificial insemination.

44 An improved flow cytometer system for isolating desired cells as described in claim 25 wherein said chemically coordinated sheath fluid source comprises a solution containing a hepes buffered medium.

45 An improved flow cytometer system for isolating desired cells as described in claim 44 wherein said cell source comprises equine sperm cells.

46 An improved flow cytometer system for isolating desired cells comprising:

- a. a cell source which supplies cells to be analyzed by the flow cytometer;
- b. a sheath fluid source which creates a sheath fluid environment for said cells which comprises a solution containing a hepes buffered medium;
- c. a nozzle through which said cells pass while subjected to said sheath fluid environment;
- d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;
- e. a cell sensing system which responds to said cells;
- f. a sorter discrimination system which acts to sort cells having a desired characteristic; and
- g. a collector into which cells having a desired characteristic are placed.

47 An improved flow cytometer system for isolating desired cells as described in claim 45 wherein said collector is used to provide a sperm for artificial insemination.

48 An improved flow cytometer system for isolating desired cells as described in claim 45 wherein said collector is used to provide a low dose of sperm for artificial insemination.

- 49 An improved flow cytometer system for isolating desired cells as described in claim 22, 23, 25, 30, 43, or 48 wherein said nozzle, oscillator, cell sensing system, and sorter discrimination system are part of a flow cytometer system and wherein said flow cytometer system comprises a high speed cell sorter.
- 5 50 An improved flow cytometer system for isolating desired cells as described in claim 49 wherein said high speed cell sorter sorts said cells to be analyzed at a rate of at least about five hundred sorts per second.
- 51 An improved flow cytometer system for isolating desired cells as described in claim 49 wherein said high speed cell sorter operates at a pressure of at least about fifty pounds per square inch.
- 10 52 An improved flow cytometer system for isolating desired cells as described in claim 30 wherein said collector comprises a container comprising a cushioning element.
- 53 An improved flow cytometer system for isolating desired cells as described in claim 52 wherein said container comprises a wide collection tube.
- 15 54 An improved flow cytometer system for isolating desired cells comprising:
- a. a cell source which supplies bovine sperm cells to be analyzed by the flow cytometer;
 - b. a chemically coordinated sheath fluid source which creates a sheath fluid environment for said cells which is contains about 2.9% sodium citrate;
 - c. a nozzle through which said cells pass while subjected to said sheath fluid environment;
 - d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;
 - e. a cell sensing system which responds to said cells;
- 20

- f. a sorter discrimination system which acts to sort cells having a desired characteristic;
- g. a collector into which cells having a desired characteristic which contains a citrate collection fluid comprising about six percent egg yolk and which is used to provide a dosage of less than about five hundred thousand sperm;

and wherein said nozzle, oscillator, cell sensing system, and sorter discrimination system are part of a flow cytometer system which sorts said cells to be analyzed at a rate of at least about five hundred sorts per second and operates at a pressure of at least about fifty pounds per square inch.

55 An improved flow cytometer system for isolating desired cells comprising:

- a. a cell source which supplies equine sperm cells to be analyzed by the flow cytometer;
- b. a chemically coordinated sheath fluid source which creates a sheath fluid environment for said cells which is contains a hepes buffered medium;
- c. a nozzle through which said cells pass while subjected to said sheath fluid environment;
- d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;
- e. a cell sensing system which responds to said cells;
- f. a sorter discrimination system which acts to sort cells having a desired characteristic;
- g. a collector into which cells having a desired characteristic which contains a collection fluid comprising a hepes buffered medium and which is used to provide a dosage of less than about ten million sperm;

and wherein said nozzle, oscillator, cell sensing system, and sorter discrimination system are part of a flow cytometer system which sorts said cells to be analyzed at a rate of at least about five hundred sorts per second and operates at a pressure of at least about fifty pounds per square inch.

56 An improved flow cytometer system for isolating desired cells comprising:

- a. a cell source which supplies cells to be analyzed by the flow cytometer;
- b. a means for minimizing the changes between a sheath fluid environment for said cells and both a pre-sort and a post-sort cell fluid environment;
- 5 c. a nozzle through which said cells pass while subjected to said sheath fluid environment;
- d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;
- e. a cell sensing system which responds to said cells;
- f. a sorter discrimination system which acts to sort cells having a desired
- 10 characteristic; and
- g. a collector into which cells having a desired characteristic are placed.

57 An improved flow cytometer system for isolating desired cells as described in claim 56 wherein said means for minimizing the changes between a sheath fluid environment for said cells and both a pre-sort and a post-sort cell fluid environment comprises said sheath fluid.

15 58 An improved flow cytometer system for isolating desired cells as described in claim 56 or 57 wherein said collector has a collector fluid and wherein said means for minimizing the changes between a sheath fluid environment for said cells and both a pre-sort and a post-sort cell fluid environment comprises said collector fluid.

59 An improved flow cytometer system for isolating desired cells comprising:

- 20 a. a cell source which supplies cells to be analyzed by the flow cytometer;
- b. a sheath fluid source which creates a sheath fluid environment for said cells;
- c. a nozzle through which said cells pass while subjected to said sheath fluid environment;
- d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;
- 25 e. a cell sensing system which responds to said cells;

- f. a sorter discrimination system which acts to sort cells having a desired characteristic; and
- g. a collector into which cells having a desired characteristic are placed which comprises a chemically coordinated collector fluid sheath fluid source which creates a collector fluid environment for said cells which is selected to be coordinated with a prior cell fluid environment.

5

60 An improved flow cytometer system for isolating desired cells as described in claim 59 wherein said collector fluid comprises a nutrient which is coordinated to balance the level of said nutrient after completion of the sorting of said cells.

10

61 An improved flow cytometer system for isolating desired cells as described in claim 60 wherein said collector fluid comprises a citrate solution containing about six percent egg yolk.

62 An improved flow cytometer system for isolating desired cells as described in claim 60 or 61 wherein said cell source comprises cells which are hyper-responsive to a chemical composition in said collector fluid environment.

15

63 An improved flow cytometer system for isolating desired cells as described in claim 59 or 60 wherein said cell source comprises sperm cells.

64 An improved flow cytometer system for isolating desired cells as described in claim 61 wherein said cell source comprises bovine sperm cells.

65 An improved flow cytometer system for isolating desired cells as described in claim 59 wherein said collector is used to provide a low dose of sperm.

20

66 An improved flow cytometer system for isolating desired cells as described in claim 65 wherein said low dose of sperm comprises a dosage of less than about ten percent of said typical dosage.

67 An improved flow cytometer system for isolating desired cells as described in claim 65 wherein said sperm cells comprise bovine sperm cells and wherein said low dose of sperm comprises a dosage of less than about five hundred thousand sperm.

68 An improved flow cytometer system for isolating desired cells as described in claim 65 wherein said sperm cells comprise bovine sperm cells and wherein said low dose of sperm comprises a dosage of less than about three hundred thousand sperm.

69 An improved flow cytometer system for isolating desired cells as described in claim 59, 64, or 65 wherein said nozzle, oscillator, cell sensing system, and sorter discrimination system are part of a flow cytometer system and wherein said flow cytometer system comprises a high speed cell sorter.

70 An improved flow cytometer system for isolating desired cells as described in claim 69 wherein said high speed cell sorter sorts said cells to be analyzed at a rate of at least about five hundred sorts per second.

71 An improved flow cytometer system for isolating desired cells as described in claim 69 wherein said high speed cell sorter operates at a pressure of at least about fifty pounds per square inch.

72 An improved flow cytometer system for isolating desired cells as described in claim 59 wherein said sheath source comprises a chemically coordinated sheath fluid source which creates a sheath fluid environment for said cells which is selected to be coordinated with both a pre-sort and a post-sort cell fluid environment.

73 An improved flow cytometer system for isolating desired cells as described in claim 72 wherein said chemically coordinated sheath fluid source comprises a solution containing about 2.9% sodium citrate.

74 An improved flow cytometer system for isolating desired cells comprising:

- a. a cell source which supplies cells to be analyzed by the flow cytometer;
- b. a sheath fluid source which creates a sheath fluid environment for said cells;
- c. a nozzle through which said cells pass while subjected to said sheath fluid environment;
- d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;
- e. a cell sensing system which responds to said cells;
- f. a sorter discrimination system which acts to sort cells having a desired characteristic; and
- g. a collector which comprises a cushioning element.

75 An improved flow cytometer system for isolating desired cells as described in claim 74 wherein said collector comprises a container comprising said cushioning element.

76 An improved flow cytometer system for isolating desired cells as described in claim 75 wherein said container comprises a wide collection tube.

77 An improved flow cytometer system for isolating desired cells as described in claim 76 wherein said wide collection tube is at least about fifteen millimeters wide.

78 An improved flow cytometer system for isolating desired cells as described in claim 75 wherein said container comprises a test tube having the physical characteristics of a stream-matched container.

- 79 An improved flow cytometer system for isolating desired cells as described in claim 75 wherein said cell source comprises cells which are mechanically delicate.
- 80 An improved flow cytometer system for isolating desired cells as described in claim 75 wherein said cell source comprises sperm cells.
- 5 81 An improved flow cytometer system for isolating desired cells as described in claim 59, 61, or 64 wherein said collector comprises a cushioning element.
- 82 An improved flow cytometer system for isolating desired cells as described in claim 81 wherein said collector comprises a container comprising said cushioning element.
- 83 An improved flow cytometer system for isolating desired cells as described in claim 82 wherein said container comprises a wide collection tube.
- 10 84 An improved flow cytometer system for isolating desired cells as described in claim 83 wherein said wide collection tube is at least about fifteen millimeters wide.
- 85 An improved flow cytometer system for isolating desired cells as described in claim 59 wherein said container comprises a test tube having the physical characteristics of a stream-matched container.
- 15 86 An improved flow cytometer system for isolating desired cells as described in claim 83 wherein said cell source comprises cells which are mechanically delicate.
- 87 An improved flow cytometer system for isolating desired cells as described in claim 86 wherein said cell source comprises sperm cells.

- 88 An improved flow cytometer system for isolating desired cells as described in claim 79 wherein said nozzle, oscillator, cell sensing system, and sorter discrimination system are part of a flow cytometer system and wherein said flow cytometer system comprises a high speed cell sorter.
- 5 89 An improved flow cytometer system for isolating desired cells as described in claim 88 wherein said high speed cell sorter sorts said cells to be analyzed at a rate of at least about five hundred sorts per second.
- 10 90 An improved flow cytometer system for isolating desired cells as described in claim 88 wherein said high speed cell sorter operates at a pressure of at least about fifty pounds per square inch.
- 15 91 An improved flow cytometer system for isolating desired cells comprising:
- a. a cell source which supplies cells to be analyzed by the flow cytometer;
 - b. a sheath fluid source which creates a sheath fluid environment for said cells;
 - c. a nozzle through which said cells pass while subjected to said sheath fluid environment;
 - d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;
 - e. a cell sensing system which responds to said cells;
 - f. a sorter discrimination system which acts to sort cells having a desired characteristic; and
 - 20 g. a collector which comprises a means for avoiding impact between said cells and said collector.
- 92 A method of sorting cells comprising the steps of :
- a. establishing a cell source which supplies cells to be sorted;

- b. chemically coordinating a sheath fluid to create a sheath fluid environment for said cells which is coordinated with both a pre-sort and a post-sort cell fluid environment;
- c. sensing a property of said cells;
- d. discriminating between cells having a desired characteristic; and
- e. collecting cells having the desired characteristic.

93 A method of sorting cells as described in claim 92 and further comprising the step of minimizing the chemical changes said cells are subjected to as a result of being subjected to said sheath fluid.

94 A method of sorting cells as described in claim 93 wherein said step of establishing a cell source comprises the step of establishing a source of non-repairing cells.

95 A method of sorting cells as described in claim 93 wherein said step of establishing a cell source comprises the step of establishing a source of sperm cells.

96 A method of sorting cells as described in claim 93 wherein said step of establishing a cell source comprises the step of establishing a source of bovine sperm cells.

97 A method of sorting cells as described in claim 93 wherein said step of establishing a cell source comprises the step of establishing a source of equine sperm cells.

98 A method of sorting cells as described in claim 95 and further comprising the step of inseminating an animal using a low dose of said sperm cells.

99 A method of sorting cells as described in claim 96 wherein said step of chemically coordinating a sheath fluid to create a sheath fluid environment for said cells which is

coordinated with both a pre-sort and a post-sort cell fluid environment comprises the step of establishing a sheath fluid which contains about 2.9% sodium citrate.

100 A method of sorting cells as described in claim 97 wherein said step of chemically
5 coordinating a sheath fluid to create a sheath fluid environment for said cells which is coordinated with both a pre-sort and a post-sort cell fluid environment comprises the step of establishing a sheath fluid which contains a hepes buffered medium.

101 A method of sorting cells as described in claim 92, 93, or 95 and further comprising the step of sorting said cells at a high speed.

102 A method of sorting cells as described in claim 101 wherein said step of sorting said cells at
10 a high speed comprises the step of subjecting said cells to a pressure of at least about fifty pounds per square inch.

103 A method of sorting cells as described in claim 95 wherein said step of collecting cells having the desired characteristic comprises the step of cushioning said cells from impact with a collection container.

15 104 A method of sorting cells as described in claim 103 wherein said step of cushioning said cells from impact with a collection container comprises the step of providing a wide opening to said container.

105 A method of sorting cells comprising the steps of:

- a. establishing a cell source which supplies cells to be sorted;
- b. establishing a sheath fluid to create a sheath fluid environment for said cells;
- c. sensing a property of said cells;
- d. discriminating between cells having a desired characteristic;
- e. collecting cells having the desired characteristic in a collector fluid; and

- f. chemically coordinating said collector fluid to create an ending collector fluid environment for said cells which is coordinated with a pre-sort fluid environment.

106 A method of sorting cells as described in claim 105 wherein said step of establishing a cell
5 source comprises the step of providing an initial nutrient for said cells and further comprising the step of providing a collection fluid nutrient for said cells and wherein said step of collecting cells having the desired characteristic in a collector fluid comprises the step of balancing said initial nutrient and said collection fluid nutrient after the completion of said step of collecting said cells.

10 107 A method of sorting cells as described in claim 106 wherein said step of collecting cells having the desired characteristic in a collector fluid comprises the step of establishing a citrate collection fluid containing about six percent egg yolk prior to commencing said step of collecting.

108 A method of sorting cells as described in claim 107 wherein said step of establishing a cell
15 source comprises the step of establishing a source of bovine sperm cells.

109 A method of sorting cells as described in claim 105 and further comprising the step of inseminating an animal using a low dose of said sperm cells.

110 A method of sorting cells comprising the steps of :

- 20 a. establishing a cell source which supplies cells to be sorted;
b. establishing a sheath fluid to create a sheath fluid environment for said cells;
c. sensing a property of said cells;
d. discriminating between cells having a desired characteristic;
e. collecting cells having the desired characteristic comprising the step of cushioning said cells from impact with a collection container.

- 111 A method of sorting cells as described in claim 110 wherein said step of cushioning said cells from impact with a collection container comprises the step of providing a wide opening to said container.
- 112 A method of sorting cells as described in claim 111 wherein said step of establishing a cell source comprises the step of establishing a source of mechanically delicate cells.
- 113 A method of sorting cells as described in claim 111 wherein said step of establishing a cell source comprises the step of establishing a source of sperm cells.
- 114 A method of sorting cells as described in claim 112 and further comprising the step of sorting said cells at a high speed.
- 115 A method of sorting cells as described in claim 114 wherein said step of sorting said cells at a high speed comprises the step of subjecting said cells to a pressure of at least about fifty pounds per square inch.
- 116 A method of producing a sexed sperm specimen comprising the step of creating said specimen using the processes according to any of claims 92, 95, 98, 101, 110, 105, 108.
- 117 A method of producing a sexed sperm specimen as described in claim 116 and further comprising the step of sorting said cells at a high speed.
- 118 A method of producing a sexed sperm specimen as described in claim 116 and further comprising the step of providing said sexed sperm specimen for inseminating an animal using a low dose of said sperm cells.

119 A method of producing a sexed sperm specimen as described in claim 117 and further comprising the step of providing said sexed sperm specimen for inseminating an animal using a low dose of said sperm cells.

120 A method of producing an animal of a desired sex comprising the step of producing said animal using the processes according to any of claims 92, 95, 98, 101, 110, 105, or 108.

121 A method of producing a sexed sperm specimen as described in claim 120 and further comprising the step of sorting said cells at a high speed.

122 A method of producing a sexed sperm specimen as described in claim 120 and further comprising the step of inseminating an animal using a low dose of said sperm cells.

123 A method of producing a sexed sperm specimen as described in claim 121 and further comprising the step of inseminating an animal using a low dose of said sperm cells.

124. A method of producing multiple, sexed embryos from a female mammal comprising:

- a. creating superovulation in said mammal to create at least two eggs comprising the step of using an ovulatory pharmaceutical to cause multiple eggs to be produced;
- b. determining a sex of a sperm cell of a male mammal;
- c. sorting according to said sex of said sperm cells;
- d. inserting at least a portion of said sorted sperm cells into said uterus after an onset of estrus; and
- e. fertilizing a plurality of said eggs to produce at least one animal of the desired sex.

125. A method of producing multiple, sexed embryos according to claim 124 wherein said creating superovulation is encouraged during the estrous cycle.

126. A method of producing multiple, sexed embryos according to claim 125 wherein said step of using a an ovulatory pharmaceutical comprises the step of injecting said ovulatory pharmaceutical in half days increments between any of days 2 and 18.
127. A method of producing multiple, sexed embryos as described in claim 126 wherein injecting said ovulatory pharmaceutical in half day increments comprises injecting at least seven injections and wherein incorporating said estrus manipulation system occurs at least on about the sixth and seventh injections.
128. A method of producing multiple, sexed embryos as described in claim 127 wherein inserting at least a portion of said sorted sperm cells into said uterus comprises inserting said sperm cells into both uterine horns of said uterus.
129. A method of producing multiple, sexed embryos as described in claim 128 wherein inserting into both uterine horns comprises inserting said sperm cells approximately between 20 to 24 hours inclusive after said onset of said estrus.
130. A method of producing multiple, sexed embryos as described in claim 124 wherein said step of using an ovulatory pharmaceutical to cause multiple eggs to be produced comprises the step of injecting a dosage of follicle stimulating hormone a plurality of times a day.
131. A method of producing multiple, sexed embryos as described in claim 130 wherein said step of creating superovulation in said mammal to create at least two eggs further comprises the step of incorporating an estrus manipulation system comprising the step of supplementing said dosage of follicle stimulant hormone with prostaglandin F-2-alpha.
132. A method of producing multiple, sexed embryos as described in claim 131 wherein injecting said dosage of follicle stimulating hormone a plurality of times a day comprises injecting said follicle stimulating hormone in approximately half day increments at a dosage level of 6, 6,

4, 4, 2, 2, 2, and 2 mg between days 9 and 12 inclusive of the estrous cycle and wherein supplementing said dosage of follicle stimulant hormone with prostaglandin F-2-alpha comprises supplementing 25 and 12.5 mg of prostaglandin F-2-alpha on the sixth and seventh dosages, respectively, of said follicle stimulating hormone.

5 133. A method of producing multiple, sexed embryos as described in claim 124 and further comprising the steps of:

- a. staining sperm cells of a male mammal;
- b. sorting according to said sex of said sperm cells comprises through the use of high speed flow cytometry; and
- 10 c. concentrating said sorted sperm cells.

134. A method of producing multiple, sexed embryos as described in claim 124 wherein inserting at least a portion of said sorted sperm cells comprises using a low dose of said sperm cells.

135. A method of producing multiple, sexed embryos as described in claim 133 wherein inserting at least a portion of said sorted sperm cells comprises using a low dose of said sperm cells.

15 136. A method of producing an animal of a desired sex comprising producing said animal using the processes as described in claims 124.

137. A method of producing an animal of a desired sex comprising producing a sexed sperm cells specimen using the processes of claim 136 further comprising sorting said cells at a high speed.

20 138. A method of producing an animal of a desired sex as described in claim 136 further comprising inseminating said animal using a low dose of said sperm cells.

139. A method of producing an animal of a desired sex as described in claim 136 further comprising chemically coordinating a sheath fluid to create a sheath fluid environment for said cells which is coordinated with both a pre-sort and a post-sort cell fluid environment comprising establishing a sheath fluid which contains about 2.9% sodium citrate.

5 140. A method of producing an animal of a desired sex as described in claim 139 wherein chemically coordinating a sheath fluid to create a sheath fluid environment for said cells which is coordinated with both a pre-sort and a post-sort cell fluid environment comprises establishing a sheath fluid which contains a herpes buffered medium.

10 141. A method of producing an animal of a desired sex as described in claim 140 further comprising collecting cells having the desired characteristic and cushioning said cells from impact with a collection container which has a wide opening.

142. A method of sorting cells comprising:

- 15
- a. establishing a cell source which supplies cells to be sorted;
 - b. chemically coordinating a sheath fluid to create a sheath fluid environment for said cells which is coordinated with both a pre-sort and a post-sort cell fluid environment;
 - c. sensing a property of said cells;
 - d. discriminating between cells having a desired characteristic; and
 - e. collecting cells having the desired characteristic.

20 143. A method of sorting cells as described in claim 142 further comprising sorting said cells at a high speed.